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Mohd Urooj
Central Research Institute of Unani Medicine, Hyderabad, India

Mohammad Ahmed Khan
Central Research Institute of Unani Medicine, Hyderabad, India

G. Thejaswini,
Central Research Institute of Unani Medicine, Hyderabad, India

Munawwar Husain Kazmi
Central Research Institute of Unani Medicine, Hyderabad, India

Gulam Mohammed Husain
Central Research Institute of Unani Medicine, Hyderabad, India

Correspondence:
Dr. Gulam Mohammed Husain
Central Research Institute of Unani Medicine, Hyderabad, India
Email: gmhusain@gmail.com

Toxicity Evaluation of a Traditional Polyherbal Unani Formulation Jawarish Shahi in Rats

Mohd Urooj, Mohammad Ahmed Khan, G. Thejaswini, Munawwar Husain Kazmi, Gulam Mohammed Husain

ABSTRACT

Jawarish Shahi (JS) is a compound polyherbal Unani pharmacopoeial formulation indicated for Khafqan (Palpitation), Nafkh-e-Shikam (Flatulence) and Waswas (Insanity; false perception and hallucinations). Jawarish Shahi contains herbs like Halela (*Terminalia chebula*), Amla (*Emblica officinalis*), Kishneez (*Coriandrum sativum*), Elaichi Khurd, (*Elettaria cardamomum*), and Bed Mushk (*Salix caprea*). The present study was carried out as per OECD 408 guidance to evaluate 90 days repeated oral dose toxicity in male and female Sprague Dawley rats. The study was performed at dose levels 1028 and 2000 mg/kg bw. No adverse effects were reported with respect to body weight, feed intake, behavior and clinical signs indicative of systemic toxicity. The expected growth pattern was observed in body weight and feed intake as compared to control group at both dose levels in male and female rats. There were few significant alterations with respect to hematology, and clinical biochemistry, however the results were within normal range thus considered toxicologically insignificant. The microscopic examination of different organ/tissue showed that no histopathological changes were observed. The findings of the study showed that No Observed Adverse Effect Level (NOAEL) for JS is greater than 2000 mg/kg body weight.

Keywords: Jawarish Shahi, Toxicity, Rat, Unani.

INTRODUCTION

The acceptability of herbal medicinal products is rising tremendously worldwide. The healthcare policies laid by the governments in different part of world eventually, promote the use of these medicines in national healthcare settings. There is a misapprehension that natural is always safe or natural originated products are devoid of side effects [1,2]. Unani medicines possess great potential to cure chronic disease condition, however, quality control and validation as per current international scientific standards for these medicine is essential [3]. It is common among people to use herbal formulation as self-medication. There is a limitation of scientific data to support the safety claims of these medicines. The under regulated quality, contamination or adulteration of these medicine can produce serious safety issues [4]. Additionally, herbal medicines are concomitantly used with conventional medicines in absence of evidence for herb-drug interaction which may produce serious concern about patient's safety [5]. In order to carry out the benefits and risk analysis of Unani formulations there is a need of safety and efficacy data.

Jawarish Shahi (JS) is a compound Unani pharmacopoeial formulation mentioned in the National Formulary of Unani Medicine, PART-I and other classical text of Unani system of medicine. Jawarish Shahi contains herbs like Halela (*Terminalia chebula*), Amla (*Emblica officinalis*), Kishneez (*Coriandrum sativum*), Elaichi Khurd, (*Elettaria cardamomum*), and Bed Mushk (*Salix caprea*). *Terminalia chebula* (Combretaceae), is one of the most important herbal ingredient of traditional systems of medicine. The fruit is regarded as king of medicine in Tibetan system. It contains a number of hydrolysable tannins which constitute around one third of its total chemical constituents. The tannins containing carboxylic acids present in *Terminalia chebula* are gallic acid, gallotannins, ellagic acid, chebulic acid and gallic acid [6]. *Phyllanthus emblica* is another common ingredient of various formulations of traditional systems of medicine. Fruits of *Phyllanthus emblica* contain high amount of minerals, gallic acid, flavonoids, polyphenols, tannins and vitamin C [7]. *Coriandrum sativum* is globally used as culinary spice in addition to having medicinal properties. Various polyphenols (phenolic acids and flavonoids) present in the fruit are reported to be responsible for its pharmacological activities. These include phenolic acids like caffeic acid, gallic acid, ferulic acid and chlorogenic acid and flavonoids like kaempferol, quercetin and luteolin [8]. *Elettaria cardamomum* known since 3000 BC is

also called queen of spices in India. It is mainly grown in Asian countries. Its main constituents are terpenoidal principles like cymene, linalool, alpha and beta pinene, limonene, alpha phellandrene, myrcene, 1,8, cineole, terpenolene, terpinene *etc.* Traditionally roots, seeds, leaves, bark and flowers have been used for the treatment of various disorders [9].

JS is recommended for treatment of Khafqan (Palpitation), Nafkh-e-Shikam (Flatulence) and Waswas (Insanity; false perception and hallucinations) [3]. Considering the widespread clinical use of this valuable Unani formulation, efforts are directed to investigate 90 days repeated dose oral toxicity of Jawarish Shahi in Sprague Dawley (SD) rats.

MATERIALS AND METHODS

Experimental animals

The animals (SD rats) of age/weight (100 ± 20 g, 6 weeks old) for the current study were obtained from the National Institute of Nutrition, Hyderabad, India. The nulliparous and non-pregnant females were included in the study. The animals were kept in polycarbonate cages in the animal housing room with temperature of 22°C ± 3°C and relative humidity of 30-70%. The SD rats were exposed to artificial 12:12 h light/dark cycle for 90 consecutive days. The experiment was performed as per current CPCSEA guidelines. The experimental protocol was approved by the Institutional Animals Ethics Committee vide Protocol No. CRIUM/IAEC/2015/01/P02 prior initiation of the study. The animals were kept under acclimatization to stabilize into new environmental conditions. Standard feed and water was provided to them [10].

Drug / Formulation

An aqueous suspension in 0.3 % CMC (<2mL/100 g body weight (bw)) was freshly prepared every day. The drug was orally administered as an aqueous suspension at the maximum volume of 2mL/100 g bw. The control animals were administered with vehicle only. The experiment was performed at two dose levels of 1028 mg/kg bw and 2000 mg/kg bw for test drug once daily for 90 consecutive days at same time each day to minimize variations.

Dose Selection

The clinical dose for JS as per Unani literature is 5-10 g/day [3]. The adult dose was decided as 10 g/day using conservative approach in consultation with Unani physicians. Therapeutic Equivalent Dose (equivalent dose in rats is 1,028 mg/kg bw per day) and 2,000 mg/kg bw per day (i.e., approximately two times of the therapeutic equivalent dose). Third dose group with higher dose of JS is not feasible due to high clinical dose which is not justified in view of limit dose of 2,000 mg/kg as per ICH guideline.

Vehicle

The vehicle for oral administration of drug was used as an aqueous suspension of 0.3% CMC (Carboxy methylcellulose).

Drug administration

0.3 % CMC aqueous suspension of JS was prepared by triturating using mortar & pestle for oral administration of test drug. The animals were given drug orally via stainless steel gavage, as per dose calculated based on body weight for a period of 90 days. The toxicity study duration of 90 days was decided based on clinical duration of the JS mentioned in Unani literature.

Experimental design

The animals of the study (Male and Female) were divide into 3 groups consist 20 animals (10 Male + 10 Female) in each group. Group I served as control treated with 0.3% Aq. CMC suspension and Group II and Group III were treated with JS at dose levels of 1,028 mg/kg bw (JS Low Dose) and 2,000 mg/kg bw (JS High Dose) respectively. The vehicle and test drug were administered orally consecutive for 90 days. The current study was performed according to the OECD test guideline-408 [11]. The study animals were checked for any general behavior changes and mortality/morbidity throughout the experiment. Clinical observation (i.e., functional observation parameters) was done to detect the presence of toxicity signs. All the experimental animals in each group were weighed weekly throughout 90 days. The record of average feed intake for both male and female in each group was maintained at weekly interval. Feed intake was calculated by weighing the amounts of feed given to a cage group and leftovers on the next day. At the end of experiment after 90 days test drug treatment, all the animals were anaesthetized using isofluorane inhalation (EZ-Anaesthesia system) under overnight fasting condition. Blood samples were collected by retro-orbital puncture in the EDTA vacutainers for estimation of hematological parameters and serum vacutainers for analysis of biochemical parameters.

Red blood cell count (RBC), white blood cell count (WBC), Hemoglobin (Hb), platelet (PLT) and hematocrit (HCT) were analyzed using fully automated hematology analyzer (Swelab). The biochemical estimation performed for the following parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, glucose, creatinine, blood urea nitrogen (BUN), total cholesterol (TC), triglycerides (TG), total protein (TP) and albumin. These parameters were analyzed using fully automatic analyzer (Erba). Serum electrolytes such as chloride, potassium and sodium were also estimated using fully automated electrolyte analyzer (Allcare).

The gross necropsy of all the animals was performed at the end of study. The macroscopic examination of organ and tissues was done. Additionally, the organ and tissue as per protocol were isolated, trimmed and weighed. The collected tissues/organ was kept in neutral buffer saline to preserve for histological examination.

Statistical analyses

The study results observed are expressed as mean ± SEM. Data comparison for each group was performed using One-way ANOVA by GraphPad prism (version 5) GraphPad Software, Inc., CA, USA. A value of $p \leq 0.05$ was considered as statistically significant.

RESULTS

Survival & Clinical Examination

No adverse effect in reference to survival of animals of both sexes were observed. No incidence of mortality was reported in JS treated male and female rats at both tested dose levels. A daily general examination and detailed weekly clinical examination was conducted which did not revealed any abnormal clinical signs in JS treated animals at doses 1,028 mg/kg bw and 2,000 mg/kg bw as compared to animals of control group**.**

Body Weight

The pattern of body weight gain after oral administration of JS at dose levels1,028 mg/kg bw and 2,000 mg/kg for 90 consecutive days did not show any abnormal changes as compared to control group. There were no significant differences in body weight of JS treated animals as compared to control group.

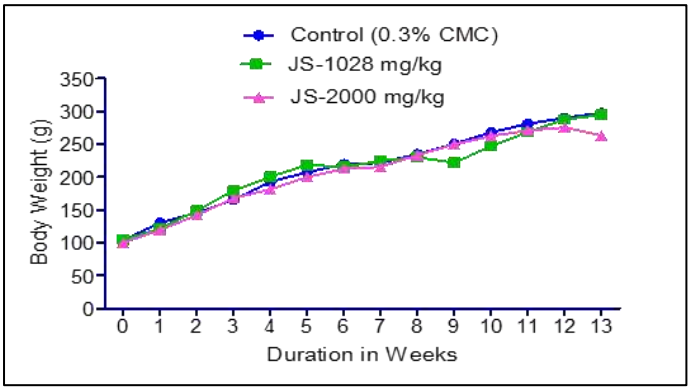

Figure 1: Average body weight of control and JS treated Male rats

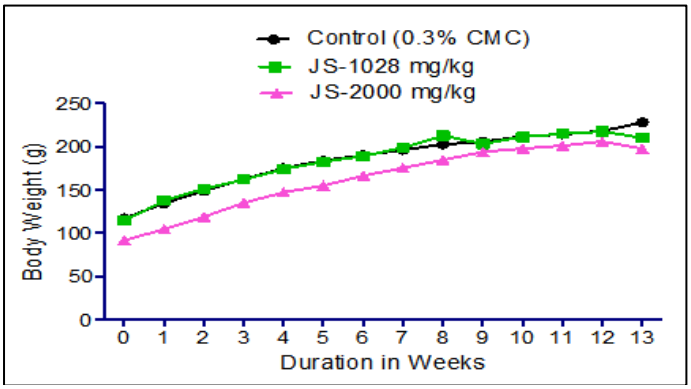

Figure 2: Average body weight of control and JS treated Female rats

3.3 Feed Consumption

No alteration was observed in reference to daily food consumption in both male and female rats as compared to control group at both tested dose level. The values of food consumption were determined weekly which was comparable to control group.

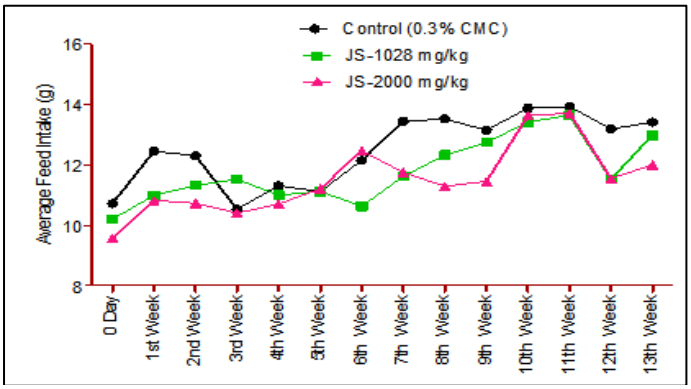

Figure-3: Average feed intake of control and JS treated Male rats

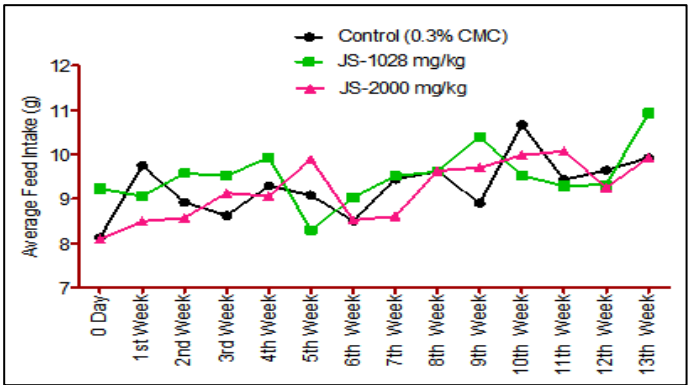

Figure 4: Average feed intake of control and JS treated Female rats

Hematology

Oral administration of JS for 90 consecutive days at dose levels 1028 and 2000 mg/kg bw showed minor alterations in few hematological parameters. Hemoglobin levels were normal in JS treated male group at both dose levels as compared to control group. However, a significant decrease in HB ($P<0.05$) in female group at dose level of 2000 mg/kg was observed though the value remained within normal physiological limits. The RBC levels were significantly reduced in male group at 1028 mg/kg ($P<0.001$) and 2000 mg/kg ($P<0.05$) and in females at dose 1028 mg/kg ($P<0.05$). There were no differences in hematocrit and platelets counts at both dose levels in any sex compared to control. Females treated with JS at 1028 and 2000 mg/kg showed no differences in WBC level compared to control, while there was a significant reduction in WBC level in males treated with JS at both dose levels ($P<0.001$) compared to control. Male and female groups treated at both doses of JS did not show any significant difference in the counts of lymphocytes, eosinophils and monocytes as compared to control group. There was significant decrease in neutrophils count in male ($P<0.05$ for both dose levels) and female rats ($P<0.01$, $P<0.05$ for low and high dose respectively) (Table 1).

Clinical Chemistry

The blood glucose levels in JS treated male and female rats at both dose levels were found normal as compared to control group, except for significant increase in JS-2000 mg/kg treated male group ($P<0.01$). In the present study the results of AST and Bilirubin tested at both dose levels were found comparable to control group. It is observed that ALT level showed dose dependent pattern of significant increase in male group while a significant decrease in female group as compared to control group. Similarly, dose independent alterations was observed for ALP level in male group at both tested dose levels whereas no alteration was observed in ALP levels in female compared to control group. Inconsistent variations observed in reference to ALT and ALP level. Moreover, the observed histological findings were similar in the experimental group and control group animals and hence may not be attributed to the administration of test drug JS (Table 2).

The level of total protein in female rats at both dose levels and in male rat at 1028 mg/kg did not show any significant difference in comparison to control group. The significant increase in total protein level ($P<0.001$) in JS treated male group was found only at high dose level as compared to control group. Creatinine level, a prognostic marker of kidney disease, did not show any significant increase in both male and female groups as compared to control group. The pattern of significant increase in the level of blood urea nitrogen (BUN) was observed in high dose male and female rats at both dose levels as compared to control group (Table 2).

Cholesterol level in the present study showed insignificant difference between JS treated male and female rats with low and high dose as compared to control group. Triglyceride level in JS treated male rats

at both dose levels was found to be comparable to control group. However, there was significant increase (P<0.01) in triglyceride level of JS treated female group at low dose while the level was normal at high dose in female as compared to control group. Notwithstanding, the triglyceride level did not exceed the reference range for SD rats. The observed values of serum electrolytes such as sodium, potassium and chloride were within the normal limits in the experimental group as compared to control groups (Table 2).

Table 1: Effect of JS on Hematology in rats vs. control

Parameter	Male			Female		
	Control (0.3% CMC)	JS-1028 mg/kg	JS-2000 mg/kg	Control (0.3% CMC)	JS-1028 mg/kg	JS-2000 mg/kg
HB (g%)	17.42±0.32	17.02±0.23	16.56±0.26	16.83±0.28	16.25±0.2162	15.7±0.2573*
RBC (Million/mm^3)	9.15±0.08	8.66±0.083**	8.81±0.088*	8.64±0.12	8.18±0.06464*	8.33±0.1136
HCT (%)	46.79±0.46	46.23±0.57	46.58±0.77	46.03±0.53	44.05±0.356	46.43±0.762
Platelet (lakhs/mm^3)	4.48±0.37	3.98±0.12	4.07±0.24	4.24±0.28	4.15±0.0654	4.5±0.2216
WBC (/mm^3)	10190±257.5	7600±431***	7430±445.5***	8280±285.9	7440±455.9	9340±440.3
Neutrophil (%)	18.1±1.01	13.9±0.88*	13.7±1.12*	17.3±0.80	12.3±1.001**	13.2±1.21*
Lymphocyte (%)	75.9±1.28	79.3±0.98	78.9±1.41	76.7±0.98	80.7±1.18	80.3±1.50
Eosinophil (%)	3.5±0.52	4.5±0.26	4.5±0.26	3.6±0.37	4.4±0.4	4.1±0.23
Monocyte (%)	2.5±0.26	2.3±0.15	2.9±0.34	2.4±0.16	2.6±0.22	2.4±0.22

(Values are expressed as Mean ± SEM; n=10/ sex; ANOVA; * p<0.05, ** = p<0.01, *** = p<0.001 vs. control)

Table 2: Effect of JS on Clinical Chemistry in rats vs. control

Parameter	Male			Female		
	Control (0.3% CMC)	JS-1028 mg/kg	JS-2000 mg/kg	Control (0.3% CMC)	JS-1028 mg/kg	JS-2000 mg/kg
Glucose (mg/dL)	94.4±4.58	92±8.28	125.7±3.23**	89.8±1.64	77.81±4.36	98.3±5.34
ALT (IU/L)	86.7±3.03	92±8.28	125.7±3.23***	77.4±5.04	69.1±7.44	53±3.84*
AST (IU/L)	137.8±8.32	131.5±4.53	145.6±14.24	125.8±4.99	127.4±7.1	140.8±8.37
Bilirubin (mg/dL)	0.131±0.009	0.155±0.010	0.1629±0.01	0.171±0.007	0.152±0.01	0.141±0.009
ALP (IU/L)	115.7±3.67	93.6±7.48*	135.3±4.56*	93.2±9.71	84±5.07	112.6±6.39
Total Protein (g/dL)	6.37±0.07	6.66±0.10	7.36±0.11***	5.97±0.05	6.91±0.08	7.53±0.11
BUN (mg/dL)	16.64±0.65	17.56±1.17	20.56±0.53**	18.53±0.71	9.4±0.84***	22.42±0.67***
Creatinine (mg/dL)	0.88±0.02	0.94±0.02	0.9±0.01	0.85±0.02	1.68±0.70	0.98±0.02
Cholesterol (mg/dL)	79.6±4.001	78.2±3.36	74.6±5.85	115.9±4.42	106±5.68	98±7.46
Triglycerides (mg/dL)	61.2±3.09	49.8±3.54	54.3±4.12	48.6±2.35	60.8±2.51**	47.1±2.90
Sodium (mmol/L)	137.3±0.47	140.3±0.78**	139.1±0.37	137.4±0.33	142.8±0.61**	138.2±1.51
Potassium (mmol/L)	4.55±0.06	4.23±0.03**	4.58±0.09	4.39±0.065	4.24±0.09	3.63±0.10***
Chloride (mmol/L)	101.2±0.57	99.3±0.94	105.6±1.43*	104.7±0.47	103.8±0.67	99±1.06***

(Values are expressed as Mean ± SEM; n=10/ sex; ANOVA; * = p<0.05, ** = p<0.01, *** = p<0.001)

Organ Weights

The oral administration of JS at dose levels 1028 and 2000 mg/kg bw did not induce any alterations in actual organ weight and relative organ weight in the following organ/tissue of Brain, Thymus, Heart, Lungs, Liver, Spleen, Adrenals, Kidney, Testis, Epididymis, Uterus and Ovaries. All the observed values of JS treated animals were comparable to control group (Figure 5 & 6).

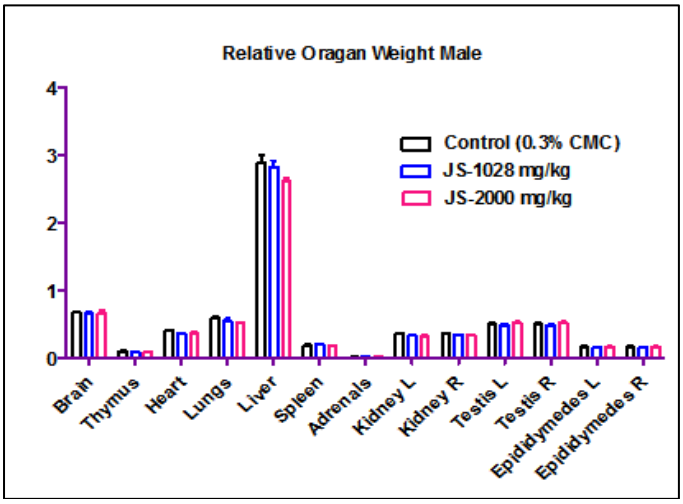

Figure 5: Relative organ weight of control and JS treated Male

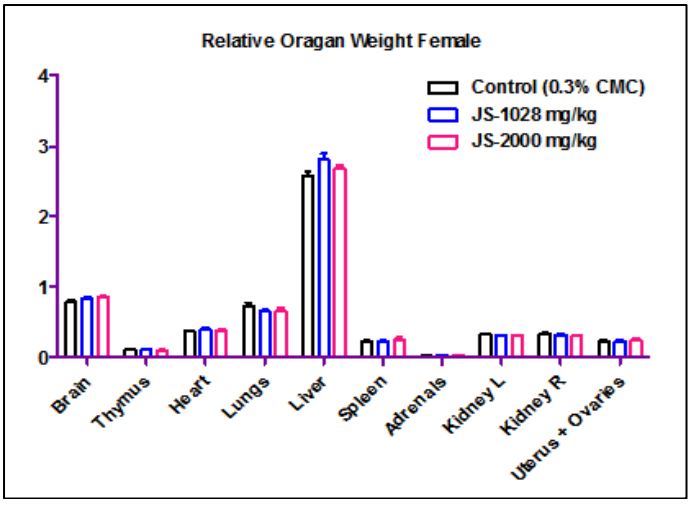

Figure 6: Relative organ weight of control and JS treated Female

Histopathology

Microscopic evaluation of isolated tissue/organs from male and female rats treated with high dose i.e., 2000 mg/kg bw did not reveal any remarkable changes. The collected organs upon histopathological examination were found histologically normal except few changes of histological significance were observed in lungs and livers of experimental and control animals. In both lungs and livers, the histological changes observed in the experimental group were also observed in the control. (Figure- 7 A-C).

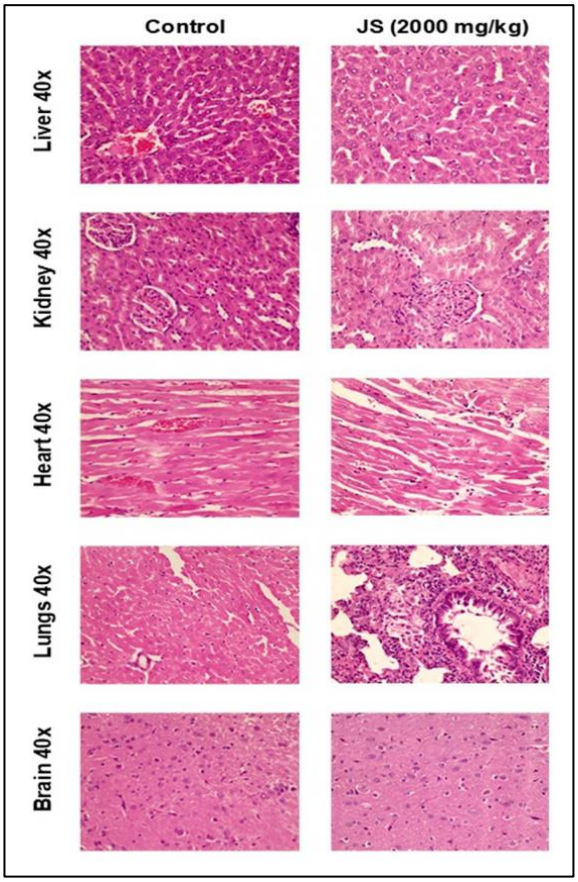

Figure 7 A: Histopathological organ section of control group vs. JS treated (2000 mg/kg bw) rats (Hematoxylin-eosin stained; magnification 40X)

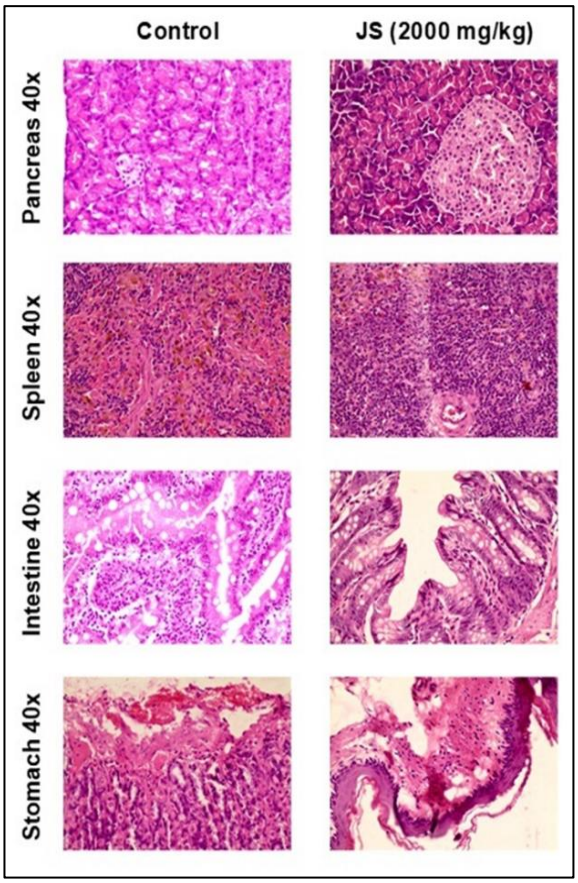

Figure 7 B: Histopathological organ section of control group vs. JS treated (2000 mg/kg bw) rats (Hematoxylin-eosin stained; magnification 40X)

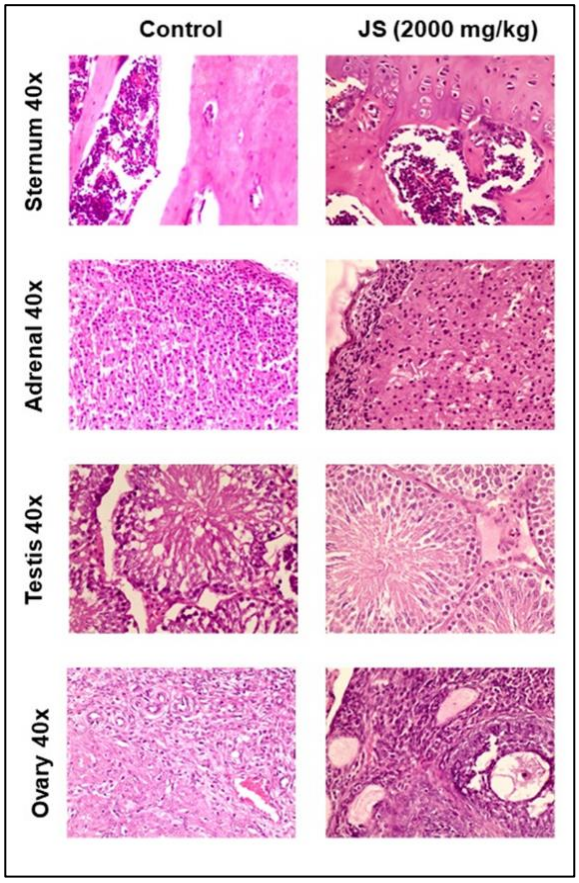

Figure 7 C: Histopathological organ section of control group vs. JS treated (2000 mg/kg bw) rats (Hematoxylin-eosin stained; magnification 40X)

DISCUSSION

The United States Food and Drug Administration (US FDA) makes no difference between herbal and conventional medicine regarding the requirement of premarketing data of safety and efficacy. There is no scientific rationale to claim plant or plant parts or derived products are intrinsically safe or beneficial [12]. Therefore, the present study was designed to evaluate the safety of traditional polyherbal Unani formulation JS by performing 90 days repeated dose oral toxicity study in SD rats. No incidence of mortality and no clinical signs indicative systemic toxicity were observed during the study duration. The pattern of body weight gain and feed intake measured weekly throughout 90 days was comparable to normal control group at both dose levels (1028 and 2000 mg/kg bw) showed consistent normal growth.

The most sensitive target of toxic compounds is hematopoietic system. It is primary index of physiological and pathological status in human and animals [13]. Based on hematological assay, most of the values in treated group were found normal in comparison to control. A few significant alterations in some hematological parameters like, Hb, RBCs, WBCs and neutrophils were observed. However, these changes were within the normal physiological range and hence cannot be considered toxicologically significant. Moreover, a chronic oral toxicity study of 180-days duration, conducted in our laboratory at a limit dose of 2,000 mg/kg bw of JS did not reveal any alteration in hematological parameters in rats [14]. *Terminalia chebula* is among major constituent of JS, hence these results may be considered in concurrence with chronic toxicity study conducted by Panunto *et al*. 2011. The results of the study revealed no significant adverse effect of *Terminalia chebula* on haematological parameters in rats [15].

Liver is the primary organ for drug metabolism. The most common adverse effect of many clinically used drugs is hepatotoxicity which is characterized by alteration in ALT, AST, ALP and bilirubin levels. Elevation in ALT and AST levels shows their leakage in blood stream indicating damage of liver parenchymal cells [16, 17]. The observation of hepatic profile in current study showed significant dose dependent alteration in ALT and ALP levels. However, alterations observed were inconsistent since, other hepatic markers were found within normal range, thus, cannot be considered toxicologically significant. Moreover, histological examination of hepatic tissue in JS treated and control group showed normal histological architecture.

Kidneys play a crucial role in drug excretion and detoxification which makes it important target for toxicological response. Exposure of kidney to high level of drug or/metabolites can cause cell damage primarily due to high blood flow, clearance and xenobiotics metabolism [18]. The major indicators for kidney damage are creatinine and serum electrolytes which were found to be within normal values in JS treated rats as compared to control. The significant alterations were observed in BUN and total protein; however, the values were within the normal physiological range therefore, considered toxicologically insignificant. Similar dose independent increase (within normal limits) in BUN level is also reported in a toxicity study conducted on bitter orange extract in SD rats [19]. Yaqin Chen *et al* conducted sub chronic toxicity study on traditional Chinese medicine "Huhezi" containing *Terminalia chebula* [20]. Our observations corroborate with the findings of the above study. Further, the chronic oral toxicity study conducted on JS did not reveal any alteration in BUN and Creatinine level at a limit dose of 2,000 mg/kg bw in rats [14]. Isolated finding of increase in total protein only in the males of high dose JS group is in consonant with studies on other herbs as reported in the literature [21]. Abnormality in lipid profile is a progressive marker for onset and progression of atherosclerosis. Dyslipidemia is common risk factor for cardiac diseases in urban population. Many herbal extracts have shown potential to normalize the altered lipid profile in humans [22]. The significant increase observed in triglycerides level in low dose JS treated female rats may not be attributed to the JS since the effect was not dose dependent. The gold standard of pathological evaluation in toxicity studies is gross necropsy followed by histopathological examination of paraffin-embedded, hematoxylin and eosin-stained tissue sections [23]. In reference to histopathological data obtained for the above-mentioned organ/tissue, no treatment related morphological alterations observed. However, changes of histopathological significance, wherever observed, showed similar characteristics in control and treated groups and were not considered treatment related.

T. chebula is one of key ingredient of JS and a repeated dose oral toxicity study of 14-day showed that the ethyl acetate-soluble portion of *T. chebula* ethanol extract showed no adverse effects at dose of 2000 mg/kg in rats [24]. However, hydrolysable tannin rich fraction of *T. chebula* fruits is reported to exhibit mild alteration in liver and kidney function in a rodent based 28 days repeated dose oral toxicity study at a dose of 1000 mg/kg [25]. Aqueous extract of another important ingredient of JS i.e., *Phyllanthus emblica* fruit did not produce any significant toxic effect following daily oral administration up to the dose of 1,200 mg/kg for 270 days in rats [26]. Similarly, hydro-methanolic extract of *Coriandrum sativum* L. is reported to be non-toxic up to 3000 mg/kg bw in mice for 28-days repeated oral administration [27]. Taken together, our findings are in consonance with the toxicological data available on individual ingredients of JS and support the safety of this valuable formulation on prolonged usage.

CONCLUSION

Based on above findings following 90 days oral administration of JS at doses 1028 and 2000 mg/kg bw, it can be concluded that JS did not show any significant adverse effects on hematology, clinical biochemistry, histopathology, body weight and feed intake. Therefore, findings of the study showed that no-observed-adverse-effect-level (NOAEL) for JS is greater than 2000 mg/kg body weight.

Conflicts of interests

The authors of manuscript do not have any conflict of interest.

Funding

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